**The Movie "Lorenzo's Oil" as a Teaching Tool  
  
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As today's student is more attuned to visual stimulation and due to the success of case-based learning, I encourage teachers to use the movie "Lorenzo's Oil" (1992, MCA Universal, 2 hrs 18 min; available at video stores or from Critic's Choice at 1800-367-7765 for $20) to illustrate many Biological principles. However, a complete understanding of the movie requires much library research.  
  
**The Disease**In the movie, Lorenzo Odone is initially portrayed as a normal, happy, healthy child whose health suddenly declines. Taking place from 1984 to 1987, the parents (Augusto Odone, played by Nick Nolte, and Michaela Odone, played by Susan Sarandon) become involved in a fight to save the life of their son. The Odones train themselves in biology to develop a treatment for their son. The sympathetic portrayal rarely fails to become an indelible memory for my students.  
  
Recently, Phil Collins has recorded a song called "Lorenzo" in his "Dance Into the Light" album. The lyrics were written by Michaela and Lorenzo.  
  
The disease portrayed in the movie is called adrenoleukodystrophy (ALD). "Leuko" is white (referring to myelin which covers and makes neurons look white) whereas "dystrophy" refers to "abnormal development." Although there are many forms, the symptoms of the form of ALD shown in the movie begin at age 5 to 12 and death occurs within a couple of years (Moser, 1997). The symptoms (dementia, loss of sight, hearing, speech, and ambulation) are believed to be due to solubilization and removal of the myelin sheath around neurons by a build up of very long chain, saturated fatty acids (VLCSFAs) in the body. Without a myelin sheath, nerve cells do not conduct action potentials. Multiple sclerosis, using a different mechanism, will also remove myelin.  
  
**Use of the Movie to Illustrate Lipid Structure**Over the past 15 years, the impact of lipid biology on our daily lives has increased and lipid research has become a hot area. The movie offers an opportunity to introduce these topics.  
  
The "very long chain" part of the VLCSFA refers to fatty acids that are 24 or 26 carbons long ("short" fatty acids have 14, 16, or 18 carbons)(Fig. 1A, B). The "saturation" of VLCSFAs refers to the fact that the carbons in the chain are saturated with hydrogens (all carbons, except the carboxyl carbon at the right end of the fatty acids in Fig. 1A and B, have at least two hydrogen atoms attached). In contrast, unsaturated carbon chains have one or more double bonds (since the carbons in the double bond have only one hydrogen attached, the carbons are not saturated with hydrogen).  
  
Due to the geometry of the cis double bond, fatty acids have a kink. Mother Nature rarely makes trans double bonds and these double bonds do not produce a kink in the carbon chain (thus, trans unsaturated fatty acids are straight like saturated fatty acids).  
  
**Kinky is Good**Triglycerides are made up of three fatty acids attached to a glycerol backbone (Fig. 1C). If one of the 3 fatty acids is an unsaturated cis fatty acid with a kink, then the triglycerides do not pack together well and the triglyceride solution is liquid at room temperature. Plants predominately make unsaturated fats and these fats are called "oils" since the unsaturated fats are liquid at room temperature. Animals predominately make saturated fats (e.g., bacon grease) that pack together well (since they have no kinks) and are solid at room temperature.  
  
Unsaturated fats obtained from plants (e.g., corn oil or olive oil) are preferred in the diet over saturated fats. High intake of saturated fats is associated with lower levels of high density lipoprotein (HDL) and higher levels of low density lipoproteins (LDL). HDL is the "good guy" since it removes cholesterol from the blood and high levels of HDL are associated with lower levels of vascular disease. So, saturated fats are associated with higher levels of blood cholesterol and plaque formation on blood vessel walls.  
  
Corn oil margarine is made by changing the double bonds in corn oil to single bonds. High pressure hydrogen gas is pumped into corn oil to force hydrogen atoms onto the carbons of the double bond in the fatty acid (a process called hydrogenation). This breaks the double bond and saturates the carbons with hydrogen. Hydrogenation removes the kinks in the fatty acid chain and allows the triglycerides to pack together well. Thus, you have a solid form that can be spread on toast. However, the industrial process also produces "trans" double bonds in the fatty acids of the triglycerides. These "trans" fats have a double bond (unsaturated) but are straight chains; they are considered as dangerous as the saturated, straight chain fats. That is, trans fats are associated with high levels of blood cholesterol and vascular disease.  
  
**But Why Are Very Long Saturated Fatty Acids Dangerous?**VLCSFAs are dangerous due to their properties. These fatty acids have a very long hydrophobic tail (the uncharged carbon chain) and a charged carboxyl group at one end.  
  
However, why would very long chain saturated fatty acids induce ALD whereas very long chain unsaturated fatty acids would not? The saturated fatty acid is straight (not crooked) and these characteristics would facilitate the ability of VLCSFAs to interact with, insert into or solubilize the hydrophobic myelin sheath. VLCSFAs may act like a "soap" to solubilize or interact with the hydrophobic molecules of the myelin sheath (micelle formation could be discussed).  
  
The exact mechanism of how VLCSFAs cause ALD symptoms is not known. As opposed to the solubilizing effect noted above, the properties of VLCSFAs would allow it to concentrate in the myelin sheath and may cause a local immune reaction that destroys the sheath. Conversely, the properties of VLCSFAs may allow it to concentrate in neural membranes to inhibit membrane function (Moser, 1995).  
  
So, fatty acids with shorter chains or with double bonds (kinks) would be less likely to insert into or solubilize the myelin sheath to cause ALD symptoms.  
  
**Use of the Movie to Illustrate the Function of Cell Organelles**Like most fatty acids and lipids (lipids being defined as any insoluble molecule), most of the steps of the synthesis of VLCSFAs are at the endoplasmic reticulum. Two carbon units are combined with shorter fatty acids to make the VLCSFAs. In the movie, Odone used a paper clip to represent the two carbon unit and he added paper clips until he produced chains of 24 or 26 carbons (12-13 paper clips).  
  
However, the very long chain fatty acids are unusual in that they are broken down by ß-oxidation in the peroxisome (short chain fatty acid breakdown occurs mostly in the mitochondrium). In ß oxidation in the peroxisome, two carbon units are removed from fatty acids (while FADH2, NADH and H202 are produced; Wanders et al., 1995). Thus, both the breakdown and the synthesis of very long chain fatty acids is by removal or addition of two carbon units.  
  
ALD is due to poor b -oxidation or breakdown of VLCSFAs in the peroxisome. Zellweger Syndrome is a disease that has symptoms similar to ALD and the syndrome occurs in infants that lack or have reduced numbers of peroxisomes.  
  
The VLCSFA "degradative" enzyme is made in the cytoplasm at free ribosomes and then shipped across the peroxisome membrane into the lumen of this organelle. The first enzyme in the degradation path is called very long chain fatty acid (or VLCFA) CoA synthase. This enzyme catalyzes the addition of acetyl-CoA to VLCFA and subsequent degradative reactions are dependent upon this first step.  
  
Recent research has shown that ALD is not due to a bad VLCFA-CoA synthase but due to a malfunctioning transporter protein that moves the VLCFA-CoA synthase across the peroxisome membrane (Valle and Gartner, 1993). The malfunctioning transporter protein is located in the membrane of the peroxisome and is a member of the ABC transporter family (ABC abbreviates ATP-binding cassette). The ABC transporters move large proteins, amino acids, and ions across membranes and are involved in drug resistance (by pumping drugs out of cells). Malfunction of a related but different ABC transporter is responsible for cystic fibrosis.  
  
**Most Inherited Diseases are Due to Expression of a Recessive Allele**The transporter molecule in ALD patients does not work well since the gene that codes for it (the recessive allele of the gene) has the "wrong" sequence of nucleotides and this produces a nonfunctional protein (with "wrong" sequence of amino acids). The normal allele would produce a normal transporter and this allele would be dominant over the defective allele. Lorenzo's mother had a normal dominant allele and a dangerous recessive allele but she did not have ALD since the dominant allele made a transporter that moved enough VLCFA-CoA synthase into the peroxisome.  
  
Although too advanced for most classes, one could discuss the discovery of the ALD gene (and topics like chromosomal mapping, PCR, stop codons, cDNA and positional cloning; see Moser et al., 1993).

**Treatment of ALD With Competitive Inhibitors Found in Lorenzo's Oil**In the movie, the first method of treatment of Lorenzo was to limit the dietary intake of VLCSFAs. Certain foods (e.g., peanut butter, spinach) contain a large amounts of VLCSFAs and Lorenzo avoided these foods. However, Lorenzo continued to show elevated levels of VLCSFAs in his blood.  
  
This provides an opportunity to recreate scenes from the movie and the scientific process of model-building to attack a question. Odone was faced with a paradox: why would limitation of the dietary VLCSFAs result in an increase in blood VLCSFAs?  
  
To understand the answer to the paradox, Odone used a "sink" model. The blood levels of VLCSFA were represented by the level of water in the bottom of a sink. Water level is dependent upon three factors: a tap on the left represented dietary VLCSFAs and it was turned off. The tap on the right was the "synthesis" tap and it was still open. This tap was open since Lorenzo's endoplasmic reticulum continued making VLCSFAs. The third factor was the drain; the breakdown of VLCSFAs by the peroxisome. ALD is caused by the drain being "clogged up" and the level of VLCSFAs was building up in the sink despite turning off the dietary tap. The Odones needed a way of shutting off the "synthesis" tap; they developed "Lorenzo's Oil" for this purpose.  
  
The next treatment for Lorenzo was found as Micheala was looking through medical references (many available through the web; see later discussion). She found an obscure 1979 reference from the Polish Journal of Biology that offered a second paradox: after feeding rats oleic acid (a short chain, unsaturated fatty acid; Fig. 1A), levels of VLCSFAs went down. At this point in the movie, Micheala suggested that the Polish researchers probably did not know about ALD and that "experts work in isolation." One function of the foundation currently run by the Odones is correct these concerns and facilitate the development of cures.  
  
They started feeding Lorenzo oleic acid and this helped lower his VLCSFAs.  
  
The paradox was: why would intake of unsaturated short chain oleic acid (only 18 carbons long) reduce the synthesis of dangerous very long chain saturated fatty acids (22-24 carbons long)?  
  
To solve the paradox, Augusto Odone (most critics thought Nick Nolte's his fake Italian accent was absurd but I did not mind) once again built a model. In the movie, the scene takes place in the library of the National Institutes of Health. Odone played the "good enzyme" that makes very long chain unsaturated fatty acids in the endoplasmic reticulum. He picks up a typical, rectangular paper clip to represent the "good" two carbon unit and adds the paper clip to the unsaturated oleic acid to make the harmless very long chain unsaturated fatty acids.  
  
His sister-in-law (Deirdre) played the "bad enzyme" that used the triangular paper clips to elongate short saturated fatty acids into the dangerous VLCSFAs. In one scene, they both took paper clips and added them to short fatty acids to increase the length of their fatty acids. The question was, why would intake of unsaturated oleic acid, used only by Odone, reduce the speed at which the sister elongates the "bad" saturated fatty acid? The scientific thinking at the time was that there were two enzymes involved.  
  
The breakthrough came when Odone suggested that only one enzyme (not two) makes both the harmless unsaturated and the dangerous saturated very long chain fatty acids.  
  
If they fed Lorenzo the unsaturated fatty acid oleic acid, then his enzyme would use oleic acid instead of equivalent saturated fatty acids to elongate into very long chain fatty acids. This is the concept of competitive inhibition (Fig. 2) (contrast it with noncompetitive or uncompetitive inhibition). There is even a dramatic point in the movie where Peter Ustinov (playing a physician) says: "This is competitive inhibition!"  
  
**A More Potent Competitive Inhibitor**The use of oleic acid lowered Lorenzo's blood VLCFA but, after 3 months, the level was still twice normal. That is, the enzyme at the endoplasmic reticulum can still pick up or utilize saturated fatty acids and add two carbon units to make VLCSFAs. A stronger competitive inhibitor was needed.  
  
Erucic acid (Fig. 1B) was chosen; it is a very long chain (22 carbons long) unsaturated fatty acid that is used by the enzyme to make longer harmless unsaturated fatty acids. With both erucic acid and oleic acid present, saturated fatty acids are not able to bind to the active site of the enzyme and are not elongated. Erucic acid may be more effective than oleic acid because of its longer chain length (it may bind to the enzyme with an affinity higher than the shorter oleic acid). It successfully lowered Lorenzo's VLCSFAs to normal levels!  
  
Lorenzo's oil is 4 parts oleic acid and one part erucic acid. Smarter students may ask why only the more potent erucic acid was not used (it is probably due to toxicity).  
  
**Sex-linked Inheritance**ALD is a sex-linked or X-linked disease (Harrell, 1997); if the Odones had a daughter, she would not have the disease.  
  
This provides an opportunity to review basic genetics. For each chromosome, there is another similar chromosome. Of these two similar or homologous chromosomes, one is from Mom and one from Dad. Genes come in two forms (alleles) and a form appears on the chromosome from Mom and another form on the chromosome from Dad. Human cells contain 22 pair of autosomal chromosomes and one pair of sex chromosomes. Autosomal homologous chromosomes are of similar size and shape but the sex chromosomes look different. Sex chromosomes (called the X and Y chromosomes) contain genes for sex determination and other traits unrelated to sex determination. The X chromosome is much larger than the Y chromosome and there are many more alleles on the X chromosome. That is, some genes that are located on the homologous sex chromosomes have only one allele (not the expected two alleles for genes found in autosomal chromosomes). The one copy of the gene is on the X chromosome and the Y chromosome lacks the second allele. For this reason, Fathers can pass on an sex chromosome allele only to their daughters, not their sons (Fig. 3).  
  
Why does sex or X- linked inheritance result in only male children having the inherited disease?  
  
To answer this, we need to note the fact that the mother has a defective gene on one of her two X chromosomes; she is without disease because she is protected by a normal gene located on the second X chromosome. The father has a normal X and Y chromosome (e.g., normal genes).  
  
There is a dramatic moment in the film when Peter Ustinov (the Doctor) tells Susan Sarandon (the mother) that "ALD is passed only through the mother" and that the defective gene is "on the female chromosome." Then the Doctor realizes the impact of what he has said and tells the mother that she should not feel guilty or say that she "caused" Lorenzo's problem.  
  
Every son has a 50% chance of inheriting the defective allele on the one X chromosome that he inherits from Mom. Ustinov, playing the Doctor, said "With each conception, there is a 50-50 chance of passing on " the defective allele. Ask students if this is true (female children will not get the disease and 50% of male children will).  
  
If a son gets the defective allele, he will have the disease because he is not protected (he will have the Y chromosome that is too small to have a normal, protective allele on it). Daughters also will inherit the bad gene 50% of the time, but they will not have the inherited disease because they will have a second, normal gene on the other X chromosome. These females with the defective gene will be "carriers" and may show slightly elevated levels of VLCSFAs (but are otherwise asymptomatic).  
  
**One Problem: Lorenzo's Oil May Not Work**Is the ending of the movie, showing many kids that have been successfully treated, a bit of propaganda or not? Most researchers have concluded that the kids never would have gotten the disease and that Lorenzo's Oil is not effective (Uziel et al., 1991; Rizzo, 1993; Aubourg et al., 1993; Odone & Odone, 1994; see also Time, Sept. 20, 1993, page 76). Dr. Moser suggests that that Lorenzo's Oil has no effect on ALD kids showing symptoms (e.g., Lorenzo) probably because the erucic acid does not enter the brain (thus, VLCSFAs still build up in the brain) (Moser, 1995). These results suggest that Lorenzo's survival is due to viability in the disease and the intense effort put forth by both Lorenzo and his parents (not the use of Lorenzo's oil).  
  
However, Lorenzo's Oil may reduce but not prevent the onset of the disease if the oil is given well before the onset of symptoms. This point is not proven and it is difficult to prove since, for ethical considerations, ongoing clinical trials are without control groups. However, Moser estimates that about 30-40% of kids in the study should have developed symptoms, and the prophylactic use of the oil may have reduced this to about 10%.  
  
RECENT REPORT: In September of 2002, Dr. Moser summarized a ten year study involving 69 boys in US and 36 boys in Europe .  He said that the data show that Lorenzo's Oil reduced the development of ALD symptoms by two-thirds!!  The use of Lorenzo's Oil was best if started before the onset of symptoms.  See:[www.kennedykrieger.org](http://www.kennedykrieger.org/" \t "_blank) for more information.  
  
**Lorenzo and ALD Treatment Today**Presently, the Odones are heading the Myelin Foundation to provide funding to researchers developing ways of remyelinating neurons (The Myelin Project, Suite 950 , 1747 Pennsylvania Ave, NW, WashingtonDC , 20006; telephone: 202-452-8994; [myelin@erols.com](mailto:myelin@erols.com)). The foundation has funded over 21 projects and an administrative cost of only 16% (many charities have up to 75% administrative costs). Recent letters from the Myelin foundation have noted that "Lorenzo is ... proud of new accomplishments (pulling his right arm across his chest)." The Foundation web site (see below) also has a touching request for nursing help for Lorenzo to keep Lorenzo's mother from a "precarious level of exhaustion."  
  
Lorenzo's oil is being given to ALD kids but new treatments of ALD are being developed. New Zealandsurgeons who have use gene therapy; they have attempted to put the good dominant allele into 2 members of a Maori tribe (where the disease is more common). An animal model for ALD was developed by the summer of 1997. The technique involved the use of "knockout" technology to remove the good allele from mice.  
  
**Ethical Questions**In the movie, the physicians are not portrayed in a good light. A movie reviewer in the journal Nature (Rosen, 1993) said that the movie portrayed "nurses as heartless, physicians pompous fools and parent support groups as mindless as a herd of sheep." Is this what is called "artistic license" to make you cheer for the Odones? What were the objections of the physicians and reasons for their objections? Were the objections reasonable? Was it appropriate for the Odones to use themselves as "guinea pigs?" and could this have done more harm than good? Doctors warned the Odones that erucic acid would cause heart problems (as it does in laboratory animals); the Odones countered with the fact that East Asians eat rapeseed oil daily.  
  
Is it appropriate for medical researchers to take the kind of chances with their patients that the Odones took with Lorenozo? Lorenzo's Oil is not without side effects. The oil reduces platelet count (thromboytopaenia)- what medical problems would this produce?  
  
Is it acceptable that we may never know the true efficacy of Lorenzo's Oil since Moser study does not have a control group?  
  
In their web site, the Odones emphasize that competition, rivalry, working in isolation, lack of funding and that publication of results often takes a year slow down progress. What methods did the Odone's use to stimulate research (answer: conduct a meeting, fund research, advertise the need for a cure for these "orphan" diseases)?  
  
What do you think about drug companies that refuse to study diseases like ALD? Their reason is that there are few patients and no large profit in developing an ALD drug. Is this an acceptable reason? The drug company may not stay in business if it does fight ALD. Should government develop new regulations that state that a percentage of profits be used to develop drugs for orphan diseases?  
  
If you were a carrier for ALD, would you have a child? Would it make a difference if you knew it was a girl or a boy? Should you be prevented from having a child by law? If the inherited disease was not sex-linked and both parents were carriers, would you have a child (calculate the odds of having a child with the inherited disease; see student questions below)?  
  
Patricia Kane, on the internet site for Carbon Based Corporation ([www.carbon.com/CBCLor2.htm](http://www.carbon.com/CBCLor2.htm" \t "_blank)), suggests use of nutrients to treat neurological disease or damage. She suggests a reason why the use of erucic acid did not turn out to be harmful as the physicians initially believed. She also goes into lipid and fatty acid structure. Is there enough evidence presented by Kane to support her statements? How do you differentiate between quackery and useful treatment of patients? Use of the scientific method to evaluate the use of fatty acids in treatment of brain injury could be discussed and students could outline experiments (how would the control group be set up or would you use one?).  
  
**Use of the Internet**Use different key words and search engines (e.g., the best one may be [www.hotbot.com](http://www.hotbot.com/" \t "_blank)) to search for more information on the internet. Examine the sites for the United Leukodystrophy Foundation or the Odone's foundation web site ([http://www.myelin.org](http://www.myelin.org/" \t "_blank)). Use the free Medline service to check for journal references on ALD or by the Odones ([http://www.ncbi.nlm.nih.gov/PubMed/](http://www.ncbi.nlm.nih.gov/PubMed/" \t "_blank)).  
  
**Questions to Ask Students**

1. Lorenzo's Oil acts at the endoplasmic reticulum or the peroxisome?
2. The movie describes the action of what two enzymes? What do the enzymes do and where are they located? (VLCSFAs are degraded in the peroxisome by a pathway that begins with the enzyme called VLCFA-CoA synthase; this is the enzyme that is missing from the peroxisomes of Lorenzo. The second enzyme is the enzyme that makes the VLCSFAs, and it is located in the endoplasmic reticulum).
3. What were the two major paradoxes in the movie? Explain them and how models and explanations were developed for both.
4. The defective allele for ALD codes for what protein? What does the protein do?
5. Does Lorenzo have one or two copies of the gene that causes ALD? Does his mother or father have one or two copies? For each person, note whether the copy is defective (recessive) or normal (dominant).
6. True or False: Most inherited diseases like ALD or cystic fibrosis are recessive diseases. What does the phrase "recessive disease" mean? (due to expression of recessive allele; inherited diseases due to dominant allele are very rare)
7. If most inherited diseases are due to the recessive allele, and many patients die before they reproduce, why does the recessive allele remain in the population? (1: in some diseases, patients of some may live long enough to reproduce and (2): the "heterozygote advantage" could be responsible. The heterozygote carrier of sickle cell anemia is resistant to malaria; heterozygtote carriers of the recessive allele for Tay-Sach's disease may be more resistant to tuberculosis, heterozygotes for the recessive cystic fibrosis allele may be protected against cholera-induced diarrhea. Thus, heterozygotes may have an advantage and would keep the dangerous recessive allele in the population).
8. Why are boys more likely to have an inherited (recessive) disease than a girl? Use a Punnett square.
9. What if Lorenzo was able to have children: what would be the odds of having a child with ALD if Lorenzo married a carrier (calculate for a female child and then a male child) or a non-carrier individual? Use a Punnett square.
10. Go through the genetics of a non-sex linked recessive disease; that is, show the genotype of parents where there are two copies of the gene involved in an inherited recessive disease. Must both parents be heterozygotes or carriers for a child to be stricken? Will both girls and boys be affected with equal probability? What is the probability of two carriers producing a normal child?
11. With ALD, why is it not possible for a female to have the genotype of homozygous recessive (from figures, aa)? Why, in some other inherited diseases, is it possible to have this genotype (aa)?
12. For a disease that is carried on non-sex chromosomes, calculate the allelic frequency of A and a and the frequency of AA and the heterozygote carrier Aa. Note that one in 100,000 children have an inherited disease; this is the frequency of "aa" in the population. What equation would you use (Hardy Weinberg Equation) and what are the assumptions for the use of this equation?
13. How could the results of Hugo Moser's study (Sept, 2002) conclude that Loenzo's Oil works yet those of Aubourg et al., 1993, conclude that it does not work?  Explain the problems with studies that use humans instead of animals such as "guinea pigs" as subjects (involve the number of subjects).